Yolk-sac Erythromyeloid Progenitors in Fracture Healing

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INTRODUCTION: Bone fractures are common injuries. However, delayed healing can be a major health issue. With aging, the pace of fracture repair slows and the chance of delayed healing increases. Strategies to improve the pace of repair and prevent non-union (the inability to heal a fracture) will substantially improve patient outcomes as well as lower health care costs. Fracture healing is a dynamic process, and many cell types from various lineages are required to restore a broken bone. Macrophages are known to play a critical role in bone healing, and these cells have several embryonic origins, yet the specific macrophage sub-populations responsible for bone healing are unknown. Previous work from our lab and others identified a reservoir of yolk-sac derived monocytes that reside in the spleen and can migrate to a fracture site, where they can differentiate into macrophages or osteoclasts. Previous work from several groups shows that when macrophages are depleted, fractures will not effectively heal. While we know that these yolk-sac derived monocytes can hone to fracture sites, their specific functions are not understood. We aim to further characterize the functions of the yolk-sac erythromyeloid progenitors and their progeny in bone healing.

METHODS: To deplete monocyte lineage yolk-sac erythromyeloid progenitors, we used genetically modified mice in which we could pulse activate cre-recombinase in monocyte lineage cells during development. We generated Cx3cr1CreERtR26T2Flm0m0 and Cx3cr1CreERtR26G0T8 mice, in which the yolk-sac derived monocytes were labeled with TdTomato, or in which Diphtheria Toxin administration will allow for the depletion of yolk-sac derived macrophages respectively. The mothers of the mice are singly injected with 4-hydroxytamoxifen (4OHT), resulting in irreversible tdTomato expression in cre-expressing cells and their progenies at E9.5 to induce the cre-recombinase. Csf1r expressing cells were also labeled as an additional control for monocytes, using the Csf1r CreERt mouse. Diphtheria Toxin-mediated cell ablation is highly sensitive and efficient at killing eukaryotic cells. A tibial osteotomy was used to induce a bone injury. The fractured and contralateral control bone were collected at different times post-surgery. Analysis of TdTomato cells was undertaken using immunofluorescence. Using micro computed tomography, we quantified changes in bone morphology, as well as quantifiable changes in bone density and volume. Statistical analyses using unpaired two-tailed t-tests will be used to compare our mutant and control conditions, that are expected to be normally distributed; quantifiable data will be represented as mean ± standard error and significance will be considered at P<0.05. Institutional IACUC approval was obtained (protocol number A133-22-07).

RESULTS: Analysis of TdTomato cells was undertaken using immunofluorescence. There were no TdTomato cells in native bone. However, following fracture, these cells were seen at the fracture site (Figs 1 and 2). Prior work showed that splenectomy, which is known to slow the pace of fracture repair, depleted the TdTomato cells at the fracture site. The density at the fracture site tends to decrease and suffers following ablation of the yolk-sac derived cells (Fig 3). This is consistent with a role for this subpopulation in fracture healing.

DISCUSSION: Here we show that yolk-sac derived monocytes hone to a bone fracture and contribute to effective fracture repair. While many cell types contribute to fracture healing, and the phenotype in depleting the cells is not as severe as that seen with depleting all macrophages, this shows that this subpopulation is important in healing. Splenectomy is known to slow fracture repair, and since this subpopulation resides in the spleen, our data likely explains the mechanism behind the slower pace of repair in this situation.

SIGNIFICANCE/CLINICAL RELEVANCE: There are currently no non-surgical treatments to aid in repair of nonunion. Therefore, strategies to improve the pace of repair capitalizing on yolk-sac derived macrophages, known to migrate to a fracture site upon injury, could provide a novel therapeutic target that could improve patient outcomes.


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Fig 1. Embryonic sources of macrophage cells in bone. Top panel shows immunohistochemistry, bottom panel shows Graphic representation of numbers of TdTomato positive cells per tibia at different ages in unfractionated bone. FITC data shows that monocytes from the hematopoietic source are present at all timepoints. Each data point, mean and 95% confidence interval shown.

Fig 2. Fetal pulse labeled Cx3cr1-tomato monocytes in bone repair. A) Immunohistochemistry of the fracture at different ages animals expressing the fetal labeled tdTomato. B) Flow analysis from the same animals. C) Graphic representation of percent of TdTomato positive cells from total counts live cells. Each data point, mean and 95% confidence interval.

Fig 3. Depletion of fetal labeled Cx3cr1-DTR mice with diphtheria toxin slows fracture healing. Representative micro-CT data showing less bone at the fracture site with Cx3cr1-DTR (yolk) compared with diphtheria toxin, but only mutant animals expressed the diphtheria receptor and would have the fetal labelled cells depleted.